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Tubercles on Legumes With and Without Cultures.

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TUBERCLES ON LEGUMES WITH AND WITHOUT CULTURES.

INTRODUCTION.

The importance of such leguminous crops as clovers, cow peas, and alfalfa, is recognized by the farmers of West Virginia. Of late, they have shown an increasing interest in the growing of alfalfa. Numerous inquiries have come to the Experiment Station about the use of artificial cultures of the tubercle-forming bacteria for inoculating alfalfa seed, and some about inoculating cow pea and clover seed. There have also been a few requests for the cultures. The cultures could not be furnished by the station, but a small amount of seed was inoculated and shipped to those who made requests, the only charge being for the seed.

It has been the policy of the station to advise those who thought of using the artificial cultures, either those furnished by the United States Department of Agriculture, or offered for sale by seedsmen, to "go slow" and try a small plat rather than a field of several acres. It seemed to be the opinion of several who made inquiries that a failure to maintain a stand of clover might be due to some extent, and of alfalfa to greater extent, to a lack of the tubercle-forming bacteria in the soil. The soils were what is known as "clover sick," and the cultures of bacteria were to be used as a remedy.

The advice given those who made inquiries and requests has been put into practice at the Station, by making a test of the artificial cultures on a small scale; at the same time, a test was made without using cultures for comparison. What has been done has been done from the farmer's, rather than from a scientific standpoint. However, it is hoped that the results obtained may have some scientific value.

FIELD TESTS IN 1904.

In the spring of 1904, the United States Department of Agriculture furnished the Station with cultures of bacteria for inoculating the seed of red clover, alfalfa, cow-pea, soy bean, garden pea, and vetch. It was arranged by Director J. H. Stewart that I should inoculate the seed and Mr. Horace Atwood, the assistant agriculturalist, should supervise the planting at the Experiment Station farm.

A few garden peas were inoculated from the garden pea culture to show a class of students how the work is done. The inoculated peas were planted in pots in the laboratory, but no results of value were obtained.

Sub-cultures were made from the remaining cultures furnished by the Department. There was considerable difference in the appearance of the sub-cultures as they gradually developed. One was covered with a thick foam after standing twenty-four hours; another became slimy; and another showed but little change after standing three days. It is probable that more care was taken in following out the instructions furnished for the preparation of the sub-cultures than would have been taken by the average farmer. Seeds of red clover, alfalfa, soy bean, cow-pea, and vetch were wet (inoculated) with the sub-cultures; they were then dried in the shade and put into sacks. Not having a culture for velvet beans, I mixed some of the sub-cultures and inoculated velvet beans with the mixture as an experiment. The velvet beans were not of the first quality, containing many immature and broken ones, which were removed. The inoculated seeds were not exposed to the sunlight, except possibly while they were being planted, and were planted at once in one-tenth-acre plats at the Station farm. At the same time, check plats were planted with seeds that had not been inoculated. Mr. Atwood directed the planting and kept records of the plats.

Legumes had been grown at the farm before, among them being clover, vetches, cow-peas, soy beans, and velvet beans, so that in all probability there was an abundance of the tubercle-forming bacteria already in the soil. However, no alfalfa had

been grown in the plats sown with alfalfa, and no cultures of any sort whatever had been used at the farm.

The season was wet, and the velvet beans did not germinate very well, so the vacant places in the rows were replanted with cow-peas. Mr. Atwood informed me, and I saw for myself when I went up to the farm, that the stand was not as good from the inoculated seed. I have accounted for this poor stand from the fact that the sub-cultures used for inoculating the seed were probably not pure, and that species of bacteria which brought about decomposition were inoculated on to the seeds. When the seeds were planted, conditions were favorable for the rapid multiplication of these bacteria as well as for the germination of the seeds, and, as a result of the action of the bacteria on the seeds, they rotted in the ground.

This matter of an inferior germinating capacity of the inoculated seeds was communicated to the division of the Department of Agriculture that furnished the cultures, and a rather caustic reply came back intimating that I did not do the work as carefully as it should have been done. Granted that it was not, yet instructions were followed as closely as possible, and if one somewhat familiar with bacteriological methods and technique could not prepare the sub-cultures and inoculate the seed, it does not seem reasonable to suppose that the average farmer would have any better success; and yet it was the farmer, and not the bacteriologist, for whom these cultures were supposed to have been prepared.

The development of the plants in the different plats was watched with considerable interest for even a slight indication of success from the effect of the inoculation. No difference was ever noticed in the growth at any time. Along in the summer, after the plants had made a good growth, Mr. Atwood and I dug up some of the plants from each plat and examined their roots for tubercles. The soil was hard, and the results were not as satisfactory as they would have been if the soil had been mellow. The following notes were taken at the time.

Soy Beans.—There are more tubercles on the plants in the in-

oculated plat and the tubercles are somewhat larger. This seems to indicate that the inoculation has been partially successful.

Velvet Beans.—There is practically no difference in the number and size of the tubercles on the two plats. The mixed subcultures were evidently not of the right kind, at least give no results.

Cow-peas.—The seed used was the Iron variety, furnished by the Department of Agriculture. No cow peas have been grown on this plat, but some had been grown for several years only a short distance away. There are plenty of tubercles on both plats.

Vetch.—Vetch was grown near these plats two years ago. There are tubercles in abundance on both plats.

Alfalfa.—A very few tubercles on the inoculated plat. The results indicate that the inoculation has been successful. There are spots of large dark green plants in each plat.

Red clover.—No striking results can be expected, for clover has been grown for years in the immediate vicinity. There is an abundance of tubercles on both plats.

All of the plats were comparatively free from weeds. The clover and alfalfa plats were clipped a few times in order to check what few weeds there were and give the other plants a chance. The vetch was allowed to self-seed. No crop was taken from the alfalfa and clover plats. Mr. Atwood furnished the following report of the yields from the soy bean, velvet bean and cow-pea combined, and the cow-pea plats in 1904.

Kind of Seed	Inoculated	Not inoculated	Gain or loss per acre from inoculation
Soy bean	180 pounds	230 pounds	loss 500 pounds
Velvet bean and cow pea	320 "	365 "	" 450 "
Cow pea	185 "	255 "	" 700 "

It will be seen from the above table, that there is no indication that the inoculations were effective. The negative yields from

the inoculated plats may be accounted for from the fact that the stand was not as good, owing to a failure of some of the inoculated seeds to germinate.

FIELD TESTS IN 1905.

Part of the tests begun in 1904 were continued in 1905. The alfalfa went through the winter in very good condition. An examination of the roots for tubercles was made by Mr. A. Lee Post, assistant bacteriologist, and myself on the twenty-first of June. We found that the dark green colored plants in both plats had tubercles on them There were a few on some of the lighter colored plants. The light colored plants were smaller than the others. Here and there in both plats were clumps of vigorous and dark colored plants with many small tubercles. Besides these clumps, there was a strip of the same kind of plants that extended through both plats, due, possibly, to a better drainage. No additional data were obtained when Mr. Atwood and I examined some of the roots on the fourth of October.

The plats where the cow peas, and the velvet beans and cow-peas combined were grown in 1904 were replanted with cow-peas and velvet beans in 1905. The seed was not inoculated, in order to see if the inoculations made the year before would give any better results the second year. On the fourth of October, Mr. Atwood and I examined some plants in each of the four plats for tubercles, and found that there were a few rather small ones on both plats of cow-peas and a few on both plats of the velvet beans. After the plants had been cut for fodder, Mr. Atwood furnished the following data of the yields from the different plats.

Kind of Seed	Seed inoculated in 1904	Seed not inoculated in 1904	Gain or loss per acre
Velvet bean	540 pounds	520 pounds	gain 200 pounds
Cow pea	515 pounds	520 pounds	loss 50 pounds

The above results indicate that the inoculations in 1904 were

favorable for the velvet beans, although a difference of only 20 pounds from a plat of one-tenth of an acre means but little. The yields of the cow peas are so nearly alike that no conclusions can be drawn from the effects of the inoculation. However, the yields of cow-peas are more than twice what they were from the same plats in 1904. In 1904 the Iron variety was planted, and in 1905, the Black Eye. The differences in yields may have been due to a difference of varieties, to a difference in the seasons, or to replanting the same ground with cow-peas. It can scarcely be attributed to any effects from the inoculations made the previous year, for negative yields were obtained from the inoculated plats both years. There may have been an increase in the effectiveness of the bacteria that were already present in the soil from one year to the next.

In the summer of 1905 I visited the farm of Mr. Jacob Post, in Harrison County, and helped dig up some alfalfa plants from plats where a test was being made with seed inoculated with cultures received from the Department of Agriculture. Mr. Post, his son who assisted in the examination at the Station farm, and I went into the field without knowing which plats were sown with inoculated seed, and, after examining plants in the several plats, we could not tell from the absence, presence, or abundance of the tubercles which plats had been sown with inoculated seed and which had not. There were some tubercles found in almost every plat. One noticeable thing was the abundance of small tubercles on a few large plants with their roots in clods of well rotted manure or of sod, indicating that material of this kind was not only favorable for the growth of the alfalfa, but also for the formation of tubercles.

No alfalfa had been grown on this farm before, but clover and cow-peas are regular crops. The roots of some cow-peas were examined and found to be thickly set with tubercles, the tubercles being very abundant in a field where several crops of cow-peas had been grown.

The National Nitro-Culture Company of West Chester, Penn., wrote to the Station, offering to furnish it with cultures for the different legumes gratis, if it cared to test them. After a con-

ference with Director Stewart, it was decided to test the cultures. Cultures for alfalfa, soy bean, cow-pea, red clover, and velvet bean were ordered. These cultures were the same as the ones the company sold for two dollars each.

Sub-cultures were made of all except soy bean, and seeds inoculated according to the directions furnished with the cultures. One of the sub-cultures was thick and slimy when used, like one of those already referred to which was made from a culture from the Department of Agriculture. The inoculated seeds were sent to the Station farm and planted in one-twentieth acre plats. Check plats were planted at the same time with seed that had not been inoculated. Some sweet clover was also planted. All the plats had been heavily fertilized with stable manure. Clover and cow-peas had been grown on them, but no alfalfa nor velvet beans.

The season was favorable for the growth of weeds, so that the plats of clover, alfalfa, and sweet clover did not do very well, even though the weeds were occasionally clipped.

Mr. Atwood and I examined some of the plants on the fourth of October, and found that there were tubercles of about the same size and abundance on both plats of velvet beans and of cow-peas., None were found on the sweet clover.

The clover and alfalfa were not examined. The cow-peas and velvet beans were harvested for fodder on the eleventh of October and the crop weighed on the eighteenth following. The cow-peas were the variety known as Black Eye, the pods being nearly all mature at the time of cutting. The yields are given in the following table.

Kind of Seed	Inoculated	Not inoculated	Gain or loss per acre from inoculation
Cow pea	410 pounds	430 pounds	loss 400 pounds
Velvet bean	390 pounds	405 pounds	loss 300 pounds

It will be seen from the table that the yields from the inoculated

plats of both cow-pea and velvet bean were negative, corresponding to the results obtained for the cow-pea, and the mixture of cow-pea and velvet bean in 1904, and for the cow-pea in 1905, when cultures from the United States Department of Agriculture were used.

The above will serve to show what results have been obtained at the Experiment Station farm from the use of artificial cultures of the tubercle-forming bacteria of the legumes furnished the Station by the United States Department of Agriculture and The National Nitro-Culture Company. In so far as results up to date show, the cultures were of little or no value. This does not necessarily mean that the cultures were worthless. No test was made to determine whether there were live tubercle-forming bacteria in the cultures. The cultures were tested in about the same way that the farmer would have tested them, and of course he would not be expected to tell whether the bacteria were alive or not before he used the cultures. His test would consist of an examination of the roots for tubercles, and possibly a comparison of the size of the plants from inoculated and uninoculated seed. If he had followed instruction, and did not find tubercles after a reasonable length of time, he would naturally infer that the cultures were worthless; and if he found tubercles of about the same size and abundance on plants from both inoculated and uninoculated seed, he could not very well tell whether the cultures were good or not. He would probably finally conclude that there were bacteria already in his soil and that cultures were not needed. Such is probably the case in most of the soils of West Virginia. There may be a few soils where artificial cultures, or soil from fields where legumes had been grown, can be used to advantage as a means for introducing the bacteria into these soils. At the request of Director Stewart, I have inoculated seed for several persons in different parts of the State, who were anixous to try growing alfalfa, and as a means of obtaining a stand of clover where for some unknown reason this has not been accomplished recently. In so far as I have been able to obtain reliable data, these, and other persons who have used the artificial cultures, have not obtained satisfactory results.

GREENHOUSE TESTS IN 1904.

For some time I have questioned the results claimed to have been obtained from inoculating the seeds of legumes with artificial cultures of the tubercle forming bacteria. Not that these results have been discredited entirely, but I wondered whether those who were making the claims might not be misinterpreting their results in part. In many instances it is known that check tests were not made. There is a possibility that the bacteria are more universally distributed than is often supposed, especially in the older states like West Virginia, where leguminous crops have been grown for many years. A number of species of wild legumes flourish almost everywhere.

Having found tubercles on wild legumes and cultivated ones, including alfalfa, where they have not been previously grown and where no inoculation had been made, I thought a test of a number of them in the same soil ought to give an indication, (1) as to whether there were tubercle-forming bacteria already in the soil, (2) their ability, if present, to produce tubercles on the roots of different legumes when grown in the soil, and, (3), to show whether there is as much difference between the bacteria found in the tubercles of different legumes as is sometimes supposed.

In every instance, I have reserved the privilege of throwing the "benefit of the doubt" in my own favor. However, it must be admitted that at times I have probably not given the bacteria and the legumes their "just due," as for instance in digging up the plants, even with the utmost care, it would be almost impossible not to break off some of the tubercles, so that a plant having but one tubercle, or at most only a few, would be considered as giving a negative result.

The soil used for this work was taken from what was once an old garden at the corner of Fayette and High streets, Morgantown, where the new theater is now building. On the eleventh of October, 1904, seeds of the following legumes were planted in small plats of this soil in the east bench in the greenhouse used for this work, and in the order named.

Red clover	Vetch	Pea
White clover	Soy bean	Red clover
Crimson clover	Cow-pea	White clover
Sweet clover	Bean (wax)	Scarlet clover
Alfalfa	Velvet bean	Sweet clover
Japanese clover	Bean (green-pod)	Alfalfa

Another lot of seeds was planted in the bench on the west side of the greenhouse somewhat later, but instead of in plats, they were planted in rows across the bench. They were planted in the following order:

Red clover	Bean (wax)	Japanese clover
Alsike clover	Cow-pea	Broad pea
White clover	Velvet bean	Sweet pea
Crimson clover	Bean (green-pod)	Vetch
Sweet clover	Pea	Soy bean
Alfalfa	Red clover	Bean (green-pod)
Japanese clover	Alsike clover	Cow-pea
Sweet pea	White clover	Velvet bean
Vetch	Scarlet clover	Bean (wax)
Soy bean	Sweet clover	Pea
	Alfalfa	

These plants were kept free from weeds and given as good care as possible. Considering the season, and the location of the greenhouse with respect to sunshine, all of the different species made a very good growth.

From time to time, a few plants were removed from the different plats or rows and the roots examined for tubercles. Without giving all the data obtained, but summarizing instead, tubercles were found on all of the different species with the exception of the soy bean. This shows that there were already present in the soil bacteria that could produce tubercles on ten genera of legumes, representing fourteen different species, without the use of artificial cultures. The cultures as offered for sale might lead one to suppose that there are as many kinds of tubercle-forming bacteria as there are legumes on which they occur. If this is true, there must have been a fairly good collection of the different kinds in the soil from that garden. In all probability several species of legumes grown in the greenhouse were never grown

in that garden. Since there were so many of these legumes with tubercles on them, one might ask why it was that there were none on the soy bean. There were tubercles on the soy beans at the Station farm from the seed that had not been inoculated.

The plats in the bench where there had been cow-peas were replanted with cow-peas. The increase in the number of bacteria in the soil from the first crop was noticeable at the time when the seedlings of the second crop were just beginning to produce the first true leaves. There were considerably more tubercles on the seedlings then than there were on any of the plants of the first crop. The same conditions have been noticed in the field, when the same legume was grown in the same soil for two or more years.

The first crop of alfalfa is often not a success. The failure to obtain a good stand and a vigorous growth has been attributed in some instances to a lack of "alfalfa bacteria" in the soil. To overcome this difficulty, it is a common practice to scatter over the field to be sown with alfalfa some soil from an old alfalfa field as a means of introducing the bacteria. The artificial cultures are sometimes used as substitutes for the soil. It has been demonstrated by Dr. C. D. Hopkins of the Illinois Experiment Station, that soil where sweet clover has grown can be used in the same way as the soil from an alfalfa field, indicating the possibility that sweet clover can be used to advantage as a sort of go-between crop for alfalfa. To see whether this may or may not be true, the plats of alfalfa and of sweet clover were "turned under" and both seeded with alfalfa. There were but few tubercles on their first crop of both alfalfa and sweet clover.

When the second crop of alfalfa had made about as much growth as it would for the first shoot, one hundred plants were dug up at random from each of the plats and it was found that ninety-eight of the plants following alfalfa had tubercles on their roots, and one hundred plants following the sweet clover had tubercles on their roots. Certainly a difference of only two per cent. is very small, so small that it is scarcely worth considering. The difference may have been due to a removal of the tubercles from the two alfalfa plants in digging. This seems to show that

the sweet clover was of no more value as a crop to precede alfalfa, in order to obtain an infection of the alfalfa with the bacteria, than the crop of alfalfa was to precede alfalfa.

GREENHOUSE TESTS IN 1905.

Not being satisfied with a single test of the sweet clover and alfalfa, another bench nine feet square, containing the same kind of soil, was prepared for a second test. Garden peas and beans, sweet peas, and broad peas had been grown in this bench a few weeks before, and it was known that there was an abundance of the tubercle-forming bacteria in the soil. The bench was divided into three equal plats. One of the outside plats was planted with alfalfa; the other with sweet clover; and the middle plat with a mixture of alfalfa and sweet clover. The sweet clover seed did not germinate as well as the alfalfa, so the stand was not as thick. No weeds were allowed to grow in the plats, and all were treated as nearly alike as possible. Whenever the main shoots of the alfalfa ceased growing, the alfalfa and the alfalfa-sweet clover plats were clipped.

At the end of the second, third and fourth month after planting, a section of each plat, containing about one square yard, was marked off and the plants dug up with great care, and the roots of the plants examined for tubercles. The results are given in the following table.

Roots examined at the end of	ALFALFA PLAT			ALFALFA–SWEET CLOVER PLAT						SWEET CLOVER PLAT		
				Alfalfa			Sweet Clover					
	Total number of plants	Number with tubercles	Percentage with tubercles	Total number of plants	Number with tubercles	Percentage with tubercles	Total number of plants	Number with tubercles	Percentage with tubercles	Total number of plants	Number with tubercles	Percentage with tubercles
2nd month	282	59	20.9	193	47	24.3	87	10	11.5	180	27	15.0
3d month	441	335	75.9	178	127	71.3	109	74	67.8	251	194	77.2
4th month	520	359	69.0	348	211	60.6	81	28	34.5	225	195	86.6
Totals	1243	753	60.5	719	383	53.5	277	112	40.4	656	416	63.4
				Alfalfa			Sweet Clover					
Totals for all plants in test				1962	1138	58.0	933	528	56.0			

From the table it will be seen that there was a gradual increase in the percentages of plants bearing tubercles as the plants became older, being most marked for the sweet clover. The percentages of plants with tubercles is probably somewhat too small, for some of the tubercles may have been broken off in removing the plants from the soil.

The clipping of the alfalfa and the alfalfa-sweet clover plats, undoubtedly reduced the activities of the plants in these plats for a while. Whether the formation of tubercles bears any direct relation to the general activities of the plant was not definitely determined, but there were indications that it does. The percentages of the plants with tubercles in the sweet clover plat that was not clipped were greater in every instance than those for the sweet clover plants in the alfalfa-sweet clover plat which were clipped. The roots of the sweet clover plants that had been

clipped at the end of the fourth month, were only about half the diameter of those that had not been clipped, and the extent of the root system was noticeable less, therefore, less exposed to infection by the bacteria scattered about through the soil.

There was a gradual increase in the number of plants in the plats after the second month, owing to the tardy germination of some of the seeds. There is also a possibility that there was not enough water used after the third month to prevent the dying of the lower roots and the disintegration of the tubercles. It will be noticed by referring to the table that the percentages are lower for the clipped plats after this time. The sweet clover plat was not clipped, so the soil was shaded by the plants and the evaporation was not so great. The plants of both alfalfa and sweet clover that had tubercles on their roots were considerably larger than those without them, showing that the bacteria which produced the tubercles had been beneficial to the plants.

Judging from the percentages of the plants with tubercles, the alfalfa was infected by the bacteria in the soil as readily as the sweet clover, the total percentages being so nearly alike that they are the same for all practical purposes. The sweet clover seems to have no advantage over alfalfa as a crop to procede alfalfa, in so far as the tubercle-forming bacteria are concerned. However, it may be possible to put the soil in better condition for a crop of alfalfa by first using a crop of sweet clover, and at a less expense than can be done with an extra crop of alfalfa.

SUMMARY.

Tests were begun at the Experiment Station farm in the spring of 1904 to determine whether the artificial cultures of the tubercle-forming bacteria were of any value for inoculating the seed of different legumes. Cultures for alfalfa, red clover, vetch, soy bean, cow-pea, and velvet bean were furnished for the different tests by the United States Department of Agriculture and the National Nitro-Culture Company. Seeds were inoculated according to the directions furnished with the cultures. Check plats were planted in all cases with seeds that had not been inoculated.

The roots of some of the plants in the different plats were examined from time to time to see if any tubercles were forming on them. Without exception, it was found that there were tubercles, sooner of later, not only on the plants from the inoculated seed, but also on those that had not been inoculated, showing that there were tubercle-forming bacteria in the soil.

The stand was not as good from the inoculated seed, showing that the inoculations had in some way prevented the germination of the seed, and the yield of fodder was consequently less. The loss in fodder (calculated on an acre basis) from the inoculated plats of cow-peas was from 400 to 700 pounds; of soy beans, 500 pounds, of velvet beans, 300 pounds. To this should be added the price of the cultures ($2 per acre) and the extra labor in preparing the sub-cultures and inoculating the seed.

Since an examination of a number of the commonly cultivated legumes, growing in fields and waste places in different parts of the State, showed that there were tubercles on their roots where it was known that cultures had not been used, a test was made to see how many would have tubercles when grown under the same conditions and in practically the same soil. Only one out of the fifteen species of legumes which were planted in this soil, did not have tubercles. This indicates that there is not as much difference between the bacteria producing the tubercles on the different genera and species of legumes as is sometimes intimated, or else this soil was richly supplied with the different kinds. While there is probably a slight difference between the bacteria occurring in the tubercles of the different legumes, any one kind seems able to adapt itself readily to several legumes, so that a soil containing those that had been associated with clover might be expected to produce some tubercles if sown with alfalfa. The few on the alfalfa would gradually increase until there would be some on most of the plants in the field. Their distribution can be hastened by plowing. Then if the field is reseeded with alfalfa there will be found plenty of tubercles on the second crop.

Fields that have not grown a crop of legumes in a number of years may not have many of the bacteria in the soil. Where no tubercles can be found in a neighborhood either on volunteer or

cultivated legumes, the bacteria can be introduced into a field either by taking soil from a field where legumes have been growing and scattering it over the field or by means of cultures. There is a possibility that weed seeds and various diseases may be introduced as well as bacteria when a soil is used. While the use of cultures is not wholly condemned, provided the bacteria in the cultures are alive, it has been, and is still, believed that they are not needed for the majority of the West Virginia soils.

In some cases, probably in most cases, the failure to obtain a stand and a profitable crop of such legumes as alfalfa, clovers, and cow-peas, is due to soil conditions, and weeds rather than to a lack of the bacteria. In a few instances a failure may have been due to insects or fungus pests that attacked the plants. Weeds are probably the cause of more failures than any one other single thing. But before the crop of legumes can be grown with their roots thickly set with tubercles, there must be a crop of bacteria grown to help produce those tubercles. It requires a little time to raise a crop of the bacteria, but they multiply very rapidly if they have a few legumes to feed upon. They can then be scattered through the soil in a field by plowing and harrowing. If now the field is re-seeded with a legume, the bacteria will soon begin to do their part toward producing a profitable crop.